found at the lower field positions expected for a furan system. The hydroxylic proton was not observed due to exchange with deuterium. The process of enolization was accompanied by the deuteration of the proton in the 2-position, resulting in the collapse of the doublet at 1·55 δ to give a singlet, and the disappearance of the quartet at 5·00 δ. A spectrum of I in DMSO-d6 was exclusively that of the keto form, and no change occurred upon storage of the solution.

The muscarinic potencies and acetylcholinesterase inhibitory activities of solutions of 4,5-dehydromuscarone (I) incubated at pH 5 at 40° in D_2O (the conditions under which the nmr data were obtained) were determined throughout the course of an enolization. The muscarinic agonist activity was determined using guinea-pig ileum in Ringer Tyrode solution at 35° gassed with 5% carbon dioxide in oxygen in the presence of 1×10^{-4}M hexamethonium bromide. The response elicited by a constant small volume of the incubate, throughout 2¼ h of enolization, was compared directly with the responses obtained from constant concentrations of acetylcholine. The acetylcholinesterase activities were determined by a potentiometric pH-stat technique using bovine erythrocyte enzyme (Sigma Chemical Co.). Results show that, while a fall in the proportion of the ketonic form present is accompanied by a reduction in muscarinic potency in a manner which would suggest that the enolic form has negligible agonist activity, the acetylcholinesterase activity is almost unaffected.

The enolization of 4,5-dehydromuscarone (I) in aqueous solution at physiological pH value supports the view of Beckett (1967) that the equivalence of muscarinic action shown by L-(+)-muscarone, D-(—)-muscarone, allomuscarone and 4,5-dehydromuscarone *can* arise from enolization, contrary to the opinion of Bollinger & Eugster (1971). The present results also indicate that it is the keto- which is the active form at muscarinic receptors and that enolization can occur before the drug reaches these receptors. That the ketonic oxygen may be of greater importance at muscarinic sites than at acetylcholinesterase sites, where it is the ring oxygen which appears to assume a greater importance, supports the findings of recent work on cyclopentane analogues of muscarine (Sundelin, Wiley & others, 1973; Gualtieri, Giannella & others, 1974; Givens & Rademacher, 1974; Melchiorre, Gualtieri & others, 1975, a,b).

March 22, 1976

REFERENCES

BECKETT, A. H. (1967). *Ann. N.Y. Acad. Sci.*, **144**, 675–688.

BOLLINGER, H. & EUGSTER, C. H. (1971). *Helv. chim. Acta*, **54**, 2704–2730.

GIVENS, R. S. & RADEMACHER, D. R. (1974). *J. medl Chem.*, **17**, 457–459.

GUALTIERI, F., GIANNELLA, M., MELCHIORRE, C. & PIGINI, M. (1974). *Ibid.*, **17**, 455–457.

MEISTER, H. (1967). *Justus Liebigs Annln Chem.*, **701**, 174–197.

MELCHIORRE, C., GUALTIERI, F., GIANNELLA, M., PIGINI, M., CINGOLANI, M. L., GAMBA, G., PIGINI, P. & ROSSINI, L. (1975a). *Farmaco, Ed. Sci.*, **30**, 287–299.

MELCHIORRE, C., GUALTIERI, F., GIANNELLA, M., PIGINI, M., CINGOLANI, M. L., GAMBA, G., PIGINI, P. & ROSSINI, L. (1975b). *Ibid.*, **30**, 300–310.

ROSENKRANZ, R. E., ALLNER, K., GOOD, R., v. PHILIPSBORN, W. & EUGSTER, C. H. (1963). *Helv. chim. Acta*, **46**, 1259–1285.

SUNDELIN, K. G. R., WILEY, R. A., GIVENS, R. S. & RADEMACHER, D. R. (1973). *J. medl Chem.*, **16**, 235–239.

Quantitative investigation of ergot growing in Argentina*

GRACIELA E. FERRARO, SILVIA L. DEBENEDETTI, JORGE D. COUSSIO†, *Departamento de Bioquímica Vegetal, Catedra de Farmacognosia, Faculead de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, Buenos Aires, Argentina*

Claviceps purpurea Tullasne (Hypocreaceae) grows in Argentina infecting *Cortaderia dioica Spartina alterniflora* (*S. maritima*) and other Gramineae (Ringuelet, 1936). In a previous communication Houssay & Hug (1918), reported the pharmacological activity or ergot infecting *C. dioica*, and Izquierdo & Liceaga (1947) reported the total alkaloid content of defatted ergot from *S. maritima*, as about 1% using the method of Allport & Jones (1941) and its biological assay Izquierdo (1948).

We now report the qualitative and quantitative analysis of samples of ergot collected in Samborombón Bay, Province of Buenos Aires, Argentina.

Extraction procedure and spectrophotometric determination have been described by Smith (1930) and Alexander & Banes (1961). Both methods with suitable modifications were used.

Extraction procedure. 5 g of powdered material was

* Part 6 in the series Alkaloids of Argentine Medicinal Plants. For part 5 see *Phytochem.*, 1975, **14**, 1785–1788.

† Correspondence.

extracted by percolation with cold light petroleum until the fat, about 30% of the sample weight, was completely removed. The material was dried at room temperature, 10% NH_4OH (4 ml) was added and after extraction in a Soxhlet with peroxide-free diethyl ether for 4 h, the ethereal fraction was placed in a stoppered flask and extracted with 1% w/v tartaric acid solution (20, 10, 10, 5, 5 ml portions). The ethyl-ether was then removed from the aqueous solution under vacuum at room temperature.

Spectrophotometry. A 1 ml fraction was taken and 4 ml of *p*-dimethylaminobenzaldehyde (R) was added and the mixture placed in an ice bath and allowed to stand for 30 min. The sample was compared spectrophotometrically at 550 nm with 1 ml of ergotamine tartrate solution similarly treated for total alkaloid content. The colour observed must not differ more than 20% from that observed in the reference tube.

For the determination of water soluble alkaloid content, 25 ml of the aqueous tartaric solution were made alkaline (pH 9) with diluted (10%) NH_4OH and extracted successively with 40, 30, 30, 20 ml portions of peroxide-free ethyl ether. The ethereal extract was washed with five portions of alkaline distilled water (pH 9). The water was discarded.

The ethereal extract was shaken with 10, 5, 5, 5 ml portions of 1% tartaric acid and 1 ml of this solution was compared spectrophotometrically with 1 ml of ergotamine tartrate, treated as described previously. The results are European ergot 0·202% total alkaloid and 0·041% water soluble alkaloids (n = 8) and for Argentinian ergot the respective values were 0·920 and 0·069% (n = 14).

Quantitative t.l.c. method. An ethereal extract was prepared as already described and made up to a volume of 50 ml. Suitable proportions were applied to thin-layer plates (SG HF254, 500 μm), running solvents benzene–chloroform–ethanol (2:4:1), running time 40 min, and ergotamine was used as a reference substance. The results are in Table 1.

Table 1. *Relative percentage of alkaloids present in Argentine ergot extracts.*

Alkaloid	Reference *RF*	Determined *RF*	Relative %
Lysergic acid	0	0	*
Ergonovine	11	11	7–9
Ergonovinine		20	*
Ergotamine	43	40	3–4
Ergosine	44	43	*
Ergotoxine	67	63	78–80
Ergotaminine	73	69	*
Ergotoxinine	81	79	12–13
Dihidroergotamine	35	32	*
Dihidroergotoxine	53	50	*

* Traces, not quantified.

The percentage of isolysergic acid derived alkaloids increases as the extract ages.

In summary, three facts emerge from this work: (i) the high yield of total alkaloids in Argentine ergot; (ii) the difference in the relative percentage of alkaloids in that there was a higher percentage of alkaloids of the ergotoxine group and very low yield of alkaloids of the ergotamine group compared with European ergots; (iii) the Argentine ergot is therefore an economic source of ergotoxine alkaloids.

We thank Mr Linsqui for botanical identification. This work was supported in part by Consejo Nacional de Investigaciones Cientificas y Técnicas 6324/74.

March 11, 1976

REFERENCES

ALEXANDER, T. G. & BANES, D. (1961). *J. pharm. Sci.*, **50**, 201–204.
ALLPORT, N. L. & JONES, N. A. (1941). *Quart. J. Pharm. Pharmac.*, **14**, 106–110.
HOUSSAY, B. A. & HUG, E. (1918). *Rev. Inst. Bact. Dpto. N. Hig.*, **1**, 617–629.
IZQUIERDO, J. A. & LICEAGA, J. (1947). *Rev. Farm.*, **10**, 412–414.
IZQUIERDO, J. A. (1948). *Ibid.*, **11**, 98–103.
RINGUELET, E. J. (1936). *Rev. Fac. Agr. La Plata*, **21**, 15–186.
SMITH, M. I. (1930). *U.S. Public Health Repts.*, **45**, Part I, 1466.